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ORIGINAL ARTICLE

Study of Common Genetic Variant S447X in Lipoprotein Lipase and Its Association with Lipids and Lipoproteins in Type 2 Diabetic Patients

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Abstract Elevated plasma triglyceride and non-esterified fatty acid concentrations may cause insulin resistance and type 2 diabetes mellitus. Lipoprotein lipase (LPL) is a rate-determining enzyme in lipid metabolism. A variant in the LPL gene has been identified which alters the penultimate amino acid Serine at 447 to a stop codon (S447X), and results in a truncated LPL molecule lacking the C-terminal dipeptide Ser–Gly. The present study was designed to evaluate the frequency of S447X variant in the LPL gene and its effect on the lipid and lipoprotein levels in type 2 diabetic subjects. The genotype frequency distributions of type 2 diabetes patients and controls were in Hardy–Weinberg equilibrium. Comparison of the genotype and allelic frequencies of S447X in subjects with type 2 diabetics compared to controls demonstrated no significant difference. In subjects with type 2 diabetics having hypertriglyceridemia (TG $\geq$ 150 mg/dl) compared to diabetics with TG level <150 mg/dl, significant difference in genotype frequency was found among these groups, while allelic frequency of X was significantly differed. Logistic regression analysis showed the negative association of LPL S447X variant with TG and VLDL cholesterol, while no association with total cholesterol, HDL cholesterol and LDL cholesterol was found. The lipid levels except for HDL cholesterol were found to be significantly lower in carriers for S447X than wild type in diabetes group. The decreased level of TG and TG rich lipoprotein in subjects with SNP S447X in LPL, predicts anti-atherogenic activity of carriers for S447X variant in general population as well as type 2 diabetic patients.

Keywords Type 2 diabetes mellitus (T2DM) · Lipoprotein lipase (LPL) · LPL S447X · Triglyceride · Hypertriglyceridemia

Introduction

Cardiovascular diseases, one of the serious microvascular complications of type 2 diabetes mellitus, play a major role in our society and remain the number one killer, irrespective of successful drug intervention. Apart from diabetes, other traditional risk factors such as smoking, blood pressure, bodyweight and lifestyle account for most of the risk of cardiovascular disease worldwide [1]. In addition, increased triglyceride (TG) levels significantly contribute to increased risk of cardiovascular disease [2, 3], whereas severe hypertriglyceridemia is also associated with increased risk of pancreatitis [4].

Lipoprotein lipase (LPL) is one of the key enzymes in the metabolism of triglyceride-rich lipoproteins and is produced in adipose tissue, skeletal muscle, and heart muscle. Activated by its cofactor apolipoprotein CII [5], LPL mediates the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL) at the luminal side of the endothelium. The generated free fatty acids are subsequently used for energy production in muscle tissue or stored as fat in adipose tissue. LPL also contributes to the high density lipoprotein (HDL) pool by shedding of phospholipids and apolipoproteins during the

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hydrolysis of these lipoproteins [6]. Besides the enzymatic activity, LPL also enhances hepatic clearance of triglyceride-rich lipoproteins by mediating receptor mediated uptake of these atherogenic lipoprotein particles [7, 8]. These metabolic changes are mainly responsible for anti-atherogenic effects of LPL.

The LPL gene locus is highly polymorphic and many single nucleotide polymorphisms (SNP) in both coding and non-coding regions, that have been used to study associations with lipids, lipoproteins, and risk for atherosclerosis. More than 100 mutations in the LPL gene have been described to date. While some mutations result in total loss of function, others only exert a moderate effect on LPL activity such as the D9N and N291S [9].

One of the LPL variant 'S447X' is associated with decreased TG and increased HDL cholesterol [11]. Data on the LPL concentration and activity due to presence of this variant have been conflicting [9–11]. A S447X mutation concerns a C to G mutation in exon 9 at position 1595. This nucleotide change introduces a premature stop codon at position 447, resulting in a mature protein that lacks the C-terminal amino acids serine and glycine. In contrast to all other LPL variants, this mutation is associated with beneficial effects on lipid homeostasis and atheroprotection. There are few reports available due to mutation in genomic DNA in some other genes (Apo AI, ACE, PAI-1, Coagulation factor VII etc.) resulting into gain of function, and most of these mutations have a protective role against cardiovascular diseases [12–14].

The present study was planned with the view of studying the occurrence and effect of LPL variant S447X on triglyceride and other lipid levels in type 2 diabetic patients (T2DM). As the effect of this variant in diabetes mellitus is not focused previously in type 2 diabetes mellitus patients in Indian population.

Materials and Methods

A case–control study was conducted at department of biochemistry, B. J. Government Medical College, Pune. 150 patients of type 2 diabetes mellitus in the period of 2011–2013 were randomly selected from out patients department of Medicine, B. J. Government Medical College, Pune, between the age group 40–60 years of either sex. Patients, who were pregnant, had angina or heart failure, renal failure, hypertension and severe concurrent illness were excluded from the study. Age and sex matched 150 healthy volunteers without any clinical and biochemical evidence of diabetes or hyperlipidemia, chronic kidney, liver diseases or thyroid disorders were selected as controls. Moreover, all the control subjects had normal fasting (<110 mg/dl) and post prandial (<140 mg/dl) plasma glucose level. The study was approved by Institutional Ethical Committee and an informed consent was obtained from all individual participants included in the study after complete explanation of procedure. Every patient was advised for at least 12–14 h overnight fasting and 3 ml of venous blood was collected in plain vacutainer and serum was separated. Serum samples were analyzed for total cholesterol, triglycerides, HDL cholesterol. 3 ml of blood was collected in EDTA vacutainer to extract DNA for analysis of LPL S447X variant.

Lipid Analysis

To study the effect of the LPL S447X variant on lipid levels in T2DM, plasma triglycerides, total cholesterol and HDL-cholesterol were measured in T2DM patients and controls. Cholesterol [15] and triglyceride [16] concentrations in serum were measured with a fully automated analyzer. HDL cholesterol [17] was determined with a turbidimetric assay. LDL and VLDL cholesterol levels were calculated according to the equation of Friedwald's et al. [18].

Genetic Analysis

Venous blood samples collected in EDTA vacutainer were subsequently used for DNA extraction by salting out method [19]. Genetic variant LPL S447X for patients and controls was determined by Polymerase chain reaction followed by restriction digestion [20]. Oligonucleotides located in flanking exon 9 of the LPL gene were used to amplify the region of interest by using forward primer, 5′-TACACTAGCAATGTCTAGGTGA-3′; and reverse primer, 5′-TCAGCTTTAGCCCAGAATGC-3′. PCR reactions were carried out in a 24-well programmable thermal cycler. The PCR was carried out in a total volume of 25 µl of reaction mixture, containing 50 ng of genomic DNA; 12.5 µl 2X master mix containing 200 µmol/l of deoxynucleotides triphosphates; 10 mmol/l Tris–HCL, pH 8.8; 1.5 mmol/l $MgCl_2$; 50 mmol/l KCl, and 1 ml/l Triton X-100; 2.5 U of Taq DNA Polymerase; and 8 nmol/L each primer. The amplification strategy was 1 cycle of denaturation for 5 min 94 °C, followed by 30 cycles of denaturation for 1 min at 94 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C, followed by final extension for 5 min at 72 °C.

Restriction Digestion

The LPL S447X mutation was detected by cutting the PCR product with the restriction endonuclease *Mnl*I. The PCR product of 488 bp contains two *Mnl*I restriction sites, of which one is polymorphic, and the other site reveals the

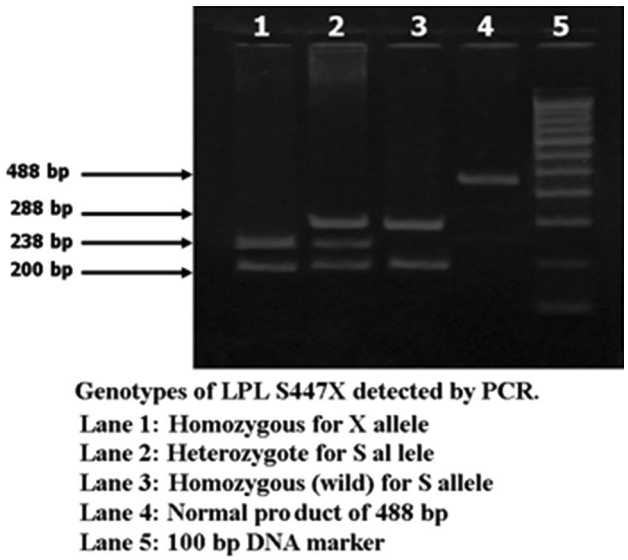

Fig. 1 Genotypes of LPL S447X detected by PCR. *Lane 1* Homozygous for X allele, *Lane 2* Heterozygote for S allele, *Lane 3* Homozygous (wild) for S allele, *Lane 4* normal product of 488 bp, *Lane 5* 100 bp DNA marker

S447X mutation. DNA fragments were separated on 3 % agarose gels and stained with ethidium bromide, and visualized by UV transilluminator. Digestion of the PCR product with 3 U *Mnl*I results in two fragments of 288 and 200 bp in case of wild type; while three fragments of 288, 238, and 200 bp for heterozygotes. The homozygous mutantion shows two fragments of 238, and 200 bp (Fig. 1). Homozygotes of the mutation were designated X447X, homozygotes of the wild type were designated S447S, and heterozygotes were designated S447X. S447X and X447X were combined and designated 447X carriers (447XC) [20].

Statistical Analysis

Deviations of the genotype distribution from expected for a population in Hardy–Weinberg equilibrium (HWE) was tested using the Chi square test. The frequencies of carriers among the T2DM patients and controls, and subgroups within T2DM were compared by using Chi square test. Frequency of the X allele is calculated and compared between study population and controls, as well as in subgroups of patients of T2DM. The continuous variables were stated as mean ± SE. These variables viz. triglyceride, total cholesterol, HDL cholesterol and LDL cholesterol, among allelic subgroups S447S and 447XC in T2DM patient groups were compared. The association between LPL S447X variant and biochemical markers was studied by logistic regression analysis. The p value of <0.05 is considered significant.

Results

In the present study a nonsense mutation of LPL, S447X in T2DM subjects as well as in controls was studied, and comparison of frequencies of LPL S447X genotype and allelic frequencies was done, and association of cardiovascular markers with S447X variant was carried in T2DM patients. No significant difference in age of T2DM patients (56.1 ± 10.73) and controls (54.5 ± 11.65) was found. The genotype frequencies of S447X variant of LPL gene of both cases and controls were calculated.

Genotype frequencies were found to be in accordance with Hardy–Weinberg equilibrium for T2DM patients ($\chi^2 = 0.0153$, $p = 0.901$) and controls ($\chi^2 = 0.7605$, $p = 0.383$). In T2DM patients, 116 (77.33 %) subjects were homozygous for the wild-type allele (SS), 32 (21.33 %) were heterozygous (SX) and 2 (1.33 %) were homozygous for the minor allele (XX). In control group, 119 (79.33 %) subjects were homozygous for the wild-type allele (SS), 28 (18.67 %) were heterozygous (SX) and 3 (2 %) subjects were homozygous for the mutant allele (XX) for SNP LPL S447X. The major allele 'S' frequencies were 0.88 and 0.89 and the minor allele 'X' frequencies were 0.12 and 0.11 in T2DM patients and controls respectively.

No significant ($p = 0.78$ and $p = 0.67$) difference in the frequencies of carriers (447XC) among T2DM subjects and controls was found in both codominant and dominant genetic models. The minor allele 'X' frequencies also did not differ significantly ($p = 0.9279$) between T2DM patients and controls. The odds ratio was calculated from the genotype frequencies among T2DM and controls and it was found to be 1.12 with 95 % confidence interval of 0.649 to 1.950 for T2DM patients. Genotype and allele frequencies of LPL S447X variant in study population and controls are shown in Table 1.

Further, T2DM patients were classified on the basis of triglyceride level into two groups, first group with triglyceride level $\geq$150 mg/dl (hypertriglyceridemia) and second group with triglyceride level $<$150 mg/dl. The genotype frequencies viz. S447S, S447X and X447X were determined in these group in type 2 diabetic patients. The genotype frequencies were 84 (87.5 %) homozygotes for wild type allele (SS), 12 (12.5 %) were heterozygotes (SX) and no any subjects were homozygotes for mutant allele (XX) in T2DM patients with hypertriglyceridemia. The genotype frequencies in T2DM patients without hypertriglyceridemia were 33 (61.11 %) were wild type (SS), 19 (35.18 %) were heterozygotes (SX) and 2 (3.7 %) were homozygotes for mutant allele (XX). The frequencies of major allele 'S' were 0.94 and 0.79 and frequencies of minor allele 'X' were 0.06 and 0.21 in T2DM patients with

Table 1 Frequencies of genotypes and alleles of S447X variant in T2DM and controls

Genetic model	Genotypes	T2DM patients (n = 150)	Controls (n = 150)	*p* value
Genotype frequencies n (%)				
Codominant	S447S	116 (77.33)	119 (79.33)	0.78
	S447X	32 (21.33)	28 (18.67)	
	X447X	2 (1.33)	3 (2)	
Dominant	S447S	116 (77.3 %)	119 (79.3 %)	0.67
	447XC	34 (22.7 %)	31 (20.7 %)	
Allele frequencies				
	S447	0.88	0.89	0.9279
	447X	0.12	0.11	

Table 2 Association of S447X mutation and hypertriglyceridemia in patients with T2DM

Genotypes	T2DM with TG ≥ 150 mg/dl (n = 96)	T2DM with TG < 150 mg/dl mg/dl (n = 54)	*p* value
Genotype frequencies n (%)			
S447S	84 (87.5)	33 (61.11)	<0.0001*
S447X	12 (12.5)	19 (35.18)	
X447X	0 (0)	2 (3.7)	
Allelic frequencies			
S447	0.94	0.79	0.0003*
447X	0.06	0.21	

* Statistically significant

and without hypertriglyceridemia respectively. The genotype frequencies differ statistically ($p < 0.0001$) among these groups on the basis of hypertriglyceridemia in T2DM patients. Also the minor allele frequencies were significantly differed among T2DM patients ($p = 0.0003$). The genotype and allelic frequencies of LPL S447X on basis of hypertriglyceridemia in T2DM patients are shown in Table 2.

Logistic regression analysis between LPL S447X variant and cardiovascular markers shows significant negative association of S447X variant with TG ($p = 0.0390$) and VLDL cholesterol ($p = 0.0364$) with Chi squared value of 32.2 and area under the ROC curve was 0.805, but no association was found of this variant with total cholesterol, HDL cholesterol and LDL cholesterol. The results of logistic regression analysis are shown in Table 3.

To study the effect of SNP S447X of LPL genotypes on demographic, cardiovascular and insulin resistance indices; we compared demographic, and biochemical parameters among genotype subgroups for this variant. The demographic and biochemical parameters were compared between genotype frequencies of LPL S447X variant within dominant genetic model (wild and carriers) in T2DM patients. We found significant decreased levels of TC ($p = 0.0002$), TG ($p < 0.0001$), and LDL-C (0.0018) adjusted for gender, smoking and alcoholic status within the carriers than the wild type for LPL S447X, while body mass index (BMI), waist to hip ratio (WHR), Fasting blood glucose, fasting insulin, homeostatic model assessment for insulin resitance (HOMA IR), HDL-C and VLDL-C did not differ statistically. The mean values with standard error (mean ± SE) of demographic and cardiovascular markers within subgroups of LPL S447X variant in patients with T2DM are depicted in Table 4.

Table 3 Logistic regression analysis for LPL S447X variant in T2DM patients

Variables	Coefficient	SE	*p* value
Triglyeceride	−3.58	1.73	0.0390*
VLDL cholesterol	−18.15	8.67	0.0364*

Discussion

LPL is a heparin-releasable enzyme bound to glycosaminoglycan compounds of the capillary endothelium in adipose and muscle tissues. LPL plays a key role in lipoprotein metabolism since it hydrolyses TG from VLDL-C and chylomicrons and also removes lipoproteins from the circulation. LPL influences the interaction between atherogenic lipoproteins and the cell surface as well as the receptors on the vascular wall, playing an important role in atherogenesis [21, 22].

Table 4 Comparison of lipid markers within subgroups of LPL S447X variant in patients with T2DM

Lipid parameters	S447S (n = 116)	447XC (n = 34)	*p* value
Age	55.49 ± 1.01	57.44 ± 2.0	0.31
BMI	27.18 ± 0.33	26.6 ± 0.42	0.48
WHR	2.22 ± 1.25	0.96 ± 0.01	0.45
Fasting blood glucose	132.05 ± 1.76	136.11 ± 2.88	0.28
Fasting insulin	10.21 ± 0.34	10.04 ± 0.62	0.78
HOMA IR	3.36 ± 0.13	3.44 ± 0.26	0.8
Total cholesterol	209.90 ± 30.45	187.5 ± 27.24	0.0002*
Triglycerides	169.0 ± 23.89	145.5 ± 13.71	<0.0001*
HDL cholesterol	43.70 ± 5.85	43.8 ± 5.25	0.914
LDL cholesterol	132.4 ± 29.11	114.50 ± 27.89	0.0018*
VLDL cholesterol	32.53 ± 0.48	32.85 ± 0.73	0.92

* Statistically significant

The gene that encodes LPL is situated on chromosome no. 8 and it is mainly expressed in adipose tissue, skeletal muscle and heart muscle [23]. The LPL gene locus is highly polymorphic, it was cloned in 1989, since then more than 100 LPL gene mutations have been identified and many SNPs in both coding and non-coding regions have been used to study associations with lipids, lipoproteins and atherosclerosis. Most of these SNPs have only mild detrimental effect on LPL function or they are markers for genetic variation elsewhere in the genome [24].

One of the naturally occurring LPL S447X variant is associated with increased lipolytic function of LPL and an anti-atherogenic lipid profile and can therefore be regarded as a gain of function mutation [24]. It is revealed that the variant alters the penultimate amino acid serine at 447 to a stop codon (S447X) resulting in a truncation of enzyme and higher expression [25].

The association of LPL S447X polymorphism with T2DM is explored in the present study. No significant association of LPL S447X polymorphism with T2DM was found as the genotype frequency findings were not significantly differed from that of controls. Further the genotype frequencies comparison in groups on the basis of presence or absence of hypertriglyceridemia (TG > 150 mg/dl), showed significant higher genotype frequency of S447X within T2DM patients without hypertriglyceridemia than with hypertriglyceridemia, this confirms the increased activity of LPL enzyme on TG with variant S447X. The similar findings were observed regarding the frequency of mutant 'X' allele which was increased significantly in T2DM patients without hypertriglyceridemia than T2DM patients with hypertriglyceridemia.

The results of the present study are in accordance with the previous study by Stanslas et al. [26] in Malaysian population, no significant difference in the genotype frequencies of LPL S447X was found in diabetics and non-diabetics. In another study from Malaysia, Vasudevan et al. [27] also reported no association of S447X variant with T2DM patients as well as essential hypertension, these results are consistent with present findings. In a sex associated review on effect of LPL S447X in familial hypertriglyceridemia, no significant difference in genotype frequencies was noted in patients and controls. The similar results were found when results were compared between males and females [28].

In a study by Lee et al. [29] from Singapore, showed the genotype frequencies of LPL S447X did not differ significantly when compared between males and females. One of the study of S447X variant in a general population showed the allele frequencies of 0.875 and 0.125 for 'S' and 'X' respectively [30]. These findings are similar to that of the present study, while in another study 'X' allele frequency was found to be 0.06, with a carrier frequency of 13 % in men with low-HDL-C level and with CHD [31].

In the present study the effect of LPL S447X on lipid phenotypes demonstrated significant decrease in TC, TG and LDL-C levels, while no significant difference in BMI, WHR, Fasting blood glucose, fasting insulin, HDL-C and HOMA IR index in carriers than wild type for LPL S447X polymorphism in T2DM patients alone. While in controls only TG level was significantly decreased in carriers than wild type for LPL S447X polymorphism.

The logistic regression analysis also established the association of decreased levels of serum TG and VLDL-C with presence of LPL S447X variant in T2DM patients. These results indicate the presence of LPL 'X' allele is associated with decreased levels of TG in both T2DM patients and control groups. In T2DM patients it is also associated with TC and LDL-C as well in T2DM patients only. This lowering effect of LPL S447X variant on TG and other lipid levels indicate a beneficial effect of this variant against CVD in T2DM patients as well as controls.

The number of researchers focused on influence of S447X variant on lipid levels in various disease conditions or in general population. Lee [29] observed increased level of HDL-C in males as well as females carriers for S447X, and significantly decrease in TG and LDL-C level in females only with carriers for S447X than wild type. A significant increase only in HDL-C was observed by Fujiwara et al. [30] in carriers than wild type for LPL S447X variant in Mima general population. Brousseau et al. [31] found no significant difference in TC, HDL-C, LDL-C and TG in carriers than wild type for this variant in men with low HDL-C level and with CHD.

Groenemeijer et al. [20] with REGRESS study group observed that, carriers of the S447X allele had significantly higher post-heparin LPL activity, higher HDL-C levels, and lower TG levels than non-carriers. Kuivenhoven et al. [32] found significantly greater number of carriers of the S447X in the high HDL-C group than in either the groups with normal or low HDL-C.

There are some studies which differ than the results of present study; these studies show no effect of this variant with plasma lipids. In the study of Hall et al. [33] and the Wandsworth Heart and Stroke Study, the levels of TG and HDL-C concentrations were not associated with LPL S447X variant in their study. In a study by Yang et al. [34], there were no significant associations found between the genotypes of S447X and T2DM as well as between the genotypes and hypertriglyceridemia in Chinese diabetes patients.

The TG lowering effect of LPL S447X variant is shown to be the anti-atherogenic, as TG is an independent risk factor for CVD. Some of the studies on activity of LPL have shown direct or indirect evidence, that increased activity of LPL was associated with the mutation S447X, which lowers plasma TG and may increase HDL-C levels in carriers of this variant [35, 36]. S447X a nonsense mutation, is known to shorten LPL by two terminal amino acids, this variant is located in the C-domain of the protein and thus may cause increased binding affinity of the truncated LPL to receptors or may affect its subunit interaction with substrate, either facilitating or otherwise affecting the formation of dimers [37, 38].

The LPL mutations influence LPL function in different ways. Catalytic activity, dimerization, secretion, and heparin binding are affected differentially and in certain combinations may change the three-dimensional structure. The S447X polymorphism has been shown that the stability and catalytic activity in the truncated form is normal, but may be high concentrations in circulation, resulting a high activity of LPL and has been considered protective, because has been associated with decreased levels of TG and increased HDL-C levels, decreasing the risk of CVD [39].

The other possible hypothesis may be, the atherogenic lipoproteins viz. VLDL-C and LDL-C are taken up by liver with the promotion by LPL via their respective receptors through acting as a ligand and/or a molecular bridge. Therefore, the lower risk of atherosclerosis has been observed in carriers of LPL S447X variant due to better clearance of atherogenic remnant lipoproteins. There could also decreased synthesis of LPL from macrophages is seen due to presence of S447X, as macrophage is one of the source of LPL. The uptake of modified LDL particles is promoted by this macrophage synthesized enzyme, so decreased synthesis leads to lowered uptake of modified LDL-C in the carriers for LPL S447X variant, thereby lowering the risk of atherosclerosis [24].

The other mechanism for the LPL S447X gain of function is altered LPL gene expression, especially at the level of translation. LPL translation is altered by catecholamines, thyroid hormone, and by poor glycemic control in diabetic subjects. In response to catecholamines in vitro, LPL translation is inhibited by the formation of an RNA binding complex containing the catalytic and regulatory subunits of protein kinase A (PKA) and A kinase anchoring protein (AKAP) which interacts with the 3′UTR of LPL mRNA. Because the translational regulation of LPL is inhibitory, and involves the 3′UTR and the region very close to the LPL S447X point mutation, the LPL S447X mutation resulted in an altered LPL mRNA which would demonstrate reduced binding to the RNA binding complex, and would therefore be better translated. An increased efficiency of LPL translation would therefore result in increased LPL activity [40].

Level of TG is under the control of activity of LPL. If the activity of LPL is enhanced, it can be beneficiary for lowering level of TG and cardiovascular risk. In various studies on animal (mice) models deficient in LPL activity and human subjects with LPL deficiency, gene therapy with AAV-LPL^{S447X} was carried out.

The permanent solution for this include gene therapy to replace the LPL gene having mutation(s) that lowers the activity of LPL enzyme, with normal one. In various studies on animal (mice) models deficient in LPL activity and human subjects with LPL deficiency, gene therapy with AAV-LPLS447X was carried out. Alipogene tiparvovec (AAV-LPL^{S447X}) is an AAV1-based gene therapy containing the LPL S447X gene construct with a constitutive expression promoter. Treatment results in histological muscle expression of LPL allied with a transient 40 % reduction in triglycerides and improvements in postprandial chylomicron triglyceride content [41].

Recently a study by Stroes et al. [42] ingested AAV1-LPL with short of two C-terminal amino acids, which is produced due to S447X variant in LPL gene in LPL deficient patients, and observed decreased level of TG.

LPL deficiency due to direct or indirect effect on LPL action, stands an etiological factor for the incidence of CVD. The basic mechanisms for LPL deficiency either include mutations in LPL gene causing decrease in LPL activity or a defect in the cofactor required for its activity or may be the abnormality in lipolytic pathway. If the effect lies in the LPL gene, the S447X variant with its beneficial effect in clearing of TG as discussed above can be used to recover LPL deficiency, with the successful gene therapy in future.

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Compliance with Ethical Standards

Conflict of interest A. A. Momin, M. P. Bankar, G. M. Bhoite declares that that have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937–52.
2. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk. 1996;3(2):213–9.
3. Krauss RM. Atherogenicity of Triglyceride-Rich Lipoproteins. The American Journal of Cardiology 1998; 81 (4, Supplement 1):13B-17B.
4. Fortson MR, Freedman SN, Webster PD III. Clinical assessment of hyperlipidemic pancreatitis. Am J Gastroenterol. 1995;90(12): 2134–9.
5. Olivecrona G, Beisiegel U. Lipid binding of apolipoprotein CII is required for stimulation of lipoprotein lipase activity against apolipoprotein CII-deficient chylomicrons. Arterioscler Thromb Vasc Biol. 1997;17(8):1545–9.
6. Taskinen MR, Nikkila EA. High density lipoprotein subfractions in relation to lipoprotein lipase activity of tissues in man–evidence for reciprocal regulation of HDL2 and HDL3 levels by lipoprotein lipase. Clin Chim Acta. 1981;112(3):325–32.
7. Merkel M, Heeren J, Dudeck W, Rinninger F, Radner H, Breslow JL, et al. Inactive lipoprotein lipase (LPL) alone increases selective cholesterol ester uptake in vivo, whereas in the presence of active LPL it also increases triglyceride hydrolysis and whole particle lipoprotein uptake. J Biol Chem. 2002;277(9):7405–11.
8. Heeren J, Niemeier A, Merkel M, Beisiegel U. Endothelial-derived lipoprotein lipase is bound to postprandial triglyceride-rich lipoproteins and mediates their hepatic clearance in vivo. J Mol Med. 2002;80(9):576–84.
9. Wittrup HH, Tybjaerg-Hansen A, Nordestgaard BG. Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease. A meta-analysis. Circulation. 1999;99(22):2901–7.
10. Kozaki K, Gotoda T, Kawamura M, Shimano H, Yazaki Y, Ouchi Y, et al. Mutational analysis of human lipoprotein lipase by carboxy-terminal truncation. J Lipid Res. 1993;34(10):1765–72.
11. Zhang H, Henderson H, Gagne SE, Clee SM, Miao L, Liu G, et al. Common sequence variants of lipoprotein lipase: standardized studies of in vitro expression and catalytic function. Biochim Biophys Acta. 1996;1302(2):159–66.
12. Franceschini G, Vecchio G, Gianfranceschi G, Magani D, Sirtori CR. Apolipoprotein AIMilano. Accelerated binding and dissociation from lipids of a human apolipoprotein variant. J Biol Chem. 1985;260(30):16321–5.
13. Margaglione M, Cappucci G, d'Addedda M, Colaizzo D, Giuliani N, Vecchione G, et al. PAI-1 plasma levels in a general population without clinical evidence of atherosclerosis: relation to environmental and genetic determinants. Arterioscler Thromb Vasc Biol. 1998;18(4):562–7.
14. Iacoviello L, Di Castelnuovo A, De Knijff P, D'Orazio A, Amore C, Arboretti R, et al. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. N Engl J Med. 1998;338(2):79–85.
15. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20(4):470–5.
16. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;19(5):476–82.
17. Warnick GR, Nauck M, Rifai N. Evolution of method for measurement of HDL cholesterol: from ultracentrifugation to homogenous assays. Clin Chem. 2001;47(9):1579–96.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.
20. Groenemeijer BE, Hallman MD, Reymer PWA, Gagné E, Kuivenhoven JA, Bruin T et al on behalf of the REGRESS Study Group. Genetic variant showing a positive interaction with β-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. Circulation. 1997; 95:2628–635.
21. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res. 1996;37:693–707.
22. Jemaa R, Fumeron F, Poirier O, Lecerf L, Evans A, Arveiler D, et al. Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study. Etude Cas Temoin sur l'Infarctus du Myocarde. J Lipid Res. 1995;36(10):2141–6.
23. Wion KL, Kirchgessner TG, Lusis AJ, Schotz MC, Lawn RM. Human lipoprotein lipase complementary DNA sequence. Science. 1987;235:1638–41.
24. Rip J, Nierman MC, Ross CJ, Jukema JW, Hayden MR, Kastelein JJP, et al. Lipoprotein lipase S447X: A naturally occurring gain-of-function mutation. Arterioscler Thromb Vasc Biol. 2006;26:1236–45.
25. Chen W, Srinivasan SR, Elkasabany A, Ellsworth DL, Boerwinkle E, Berenson GS. Influence of lipoprotein lipase serine 447 stop polymorphism on tracking of triglycerides and HDL cholesterol from childhood to adulthood and familial risk of coronary artery disease: the Bogalusa heart study. Atherosclerosis. 2001;159(2):367–73.
26. Stanslas J, Vasudevan NR, Ismail P, Shamsudin, Zamanian M: Lipoprotein lipase gene polymorphism (S447X) is not associated

with type 2 diabetes mellitus patients among Malaysian population. http://www.persatuangenetikmalaysia.com/files/congress07/04Poster14.pdf.
27. Vasudevan R, Ismail P, Stanslas J, Shamsudin N. Analysis of three genetic polymorphisms in Malaysian essential hypertensive and type 2 diabetic subjects. Afr J Biotechnol. 2009;8(10):2069–75.
28. Anagnostopoulou KK, Kolovou GD, Kostakou PM, Mihas C, Hatzigeorgiou G, Marvaki C, et al. Sex-associated effect of CETP and LPL polymorphisms on postprandial lipids in familial hypercholesterolaemia. Lipids Health Dis. 2009;8:24.
29. Lee J, Tan CS, Chia KS, Tan CE, Chew SK, Ordovas JM, et al. The lipoprotein lipase S447X polymorphism and plasma lipids: interactions with APOE polymorphisms, smoking, and alcohol consumption. J Lipid Res. 2004;45(6):1132–9.
30. Fujiwara S, Kotani K, Sano Y, Matsuoka Y, Tsuzaki K, Komichi M, et al. S447X polymorphism in the lipoprotein lipase gene and the adiponectin level in the general population: Results from the Mima study. J Atheroscler Thromb. 2008;16(3):188–93.
31. Brousseau ME, Goldkamp AL, Collins D, Demissie S, Connolly AC, Cupples LA, et al. Schaefer Polymorphisms in the gene encoding lipoprotein lipase in men with low HDL-C and coronary heart disease: the veterans affairs HDL intervention trial. J Lipid Res. 2004;45:1885–91.
32. Kuivenhoven JA, Groenemeyer BE, Boer JMA, Reymer PWA, Berghuis R, Bruin T, et al. S447X mutation in lipoprotein lipase is associated with elevated HDL cholesterol levels in 295Normolipidemic males. Arterioscler Thromb Vasc Biol. 1997;17:595–9.
33. Hall S, Talmud PJ, Cook DG, Wicks PD, Rothwell MJ, Strazzullo P, et al. Frequency and allelic association of common variants in the lipoprotein lipase gene in different ethnic groups: the Wandsworth Heart and Stroke Study. Genet Epidemiol. 2000;18:203–16.
34. Yang T, Pang CP, Tsang MW, Lam CW, Poon PMK, Chan LYS, et al. Pathogenic mutations of the lipoprotein lipase gene in Chinese patients with hypertriglyceridemic type 2 diabetes. Hum Mutat. 2003;21(4):453.
35. Mailly F, Tugrul Y, Reymer PW, Bruin T, Seed M, Groenemeyer BF, et al. A common variant in the gene for lipoprotein lipase (Asp9 → Asn). Functional implications and prevalence in normal and hyperlipidemic subjects. Arterioscler Thromb Vasc Biol. 1995;15:468–78.
36. Gerdes C, Fisher RM, Nicaud V, Boer J, Humphries SE, Talmud PJ, et al. Lipoprotein lipase variants D9 N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations: studies in the fasting and postprandial states: the European Atherosclerosis Research Studies. Circulation. 1997;96:733–40.
37. Hata A, Robertson M, Emi M, Lalouel JM. Direct detection and automated sequencing of individual alleles after electrophoretic strand separation: identification of a common nonsense mutation in exon 9 of the human lipoprotein lipase gene. Nucleic Acids Res. 1990;18:5407–11.
38. Hans HW, Anne TH, Børge GN. Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease a meta-analysis. Circulation. 1999;99:2901–7.
39. Muñoz-Barriosab S, Guzmán-Guzmána IP, Muñoz-Valleb JF, Salgado-Bernabéa AB, Salgado-Goytiaa L, Parra-Rojasa I. Association of the HindIII and S447X polymorphisms in LPL gene with hypertension and type 2 diabetes in Mexican families. Dis Markers. 2012;33:313–20.
40. Ranganathan G, Unal R, Pokrovskaya ID, Tripathi P, Rotter JI, Goodarzi MO, et al. The lipoprotein lipase (LPL) S447X gain of function variant involves increased mRNA translation. Atherosclerosis. 2012;221(1):143–7.
41. Wierzbicki AS, Viljoen A. Alipogene tiparvovec: gene therapy for lipoprotein lipase deficiency. Expert Opin Biol Ther. 2013;13(1):7–10.
42. Stroes ES, Nierman MC, Meulenberg JJ, Franssen R, Twisk J, Henny CP, et al. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. Arterioscler Thromb Vasc Biol. 2008;28(12):2303–4.